and selective cPKC activation by cell-permeable cPKC activator peptide mimicked this effect. A mutation in a putative PKC phosphorylation site in the auxiliary subunit KCNE1 (S102A) specifically abolished the voltage shift by α_1-AR stimulation or cPKC activation, suggesting cPKC phosphorylation of KCNE1(S102) increases IKs current through facilitation in voltage dependence of activation. LQT1 associated mutations at the voltage activation domain (S1-S5) of KCNQ1 subunit, also impaired cPKC regulation of the IKs channel independently of β-AR regulation, suggesting that the interactions between the KCNE1 and the voltage-gating domain of KCNQ1 is crucial for cPKC regulation. In conclusion, our study indicates that the voltage activation of IKs by cPKC isoform is important in the IKs channel regulation under adrenergic stimulation and that impairment of this regulation may be linked to LQT1 clinical phenotype.

PLATFORM AF: Protein Assemblies

1082-Plat
Conical Tomography: A Simple Method to Study Proteins in Cells at High Resolution
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Despite advances in molecular biology and genetics, the location of thousand of proteins in cells remains undetermined. The principal problems are their small dimensions and their capacity to form large assemblies by associating either with themselves or other proteins. We took advantage of the tendency to form aggregates and developed a simple method that describe the three-dimensional structure of these assemblies in cells at high resolution (2-3nm) and in three-dimensions. As a proof of principle, we studied the alphaA-crystalline, a small chaperone that plays an important role in lens transparency and cataract formation. To identify the assemblies containing the chaperone, lens tissues labeled with primary anti-alphaA-crystalline and probed with both 2nm and 5nm diameter gold particle conjugates were reconstructed by conical tomography. First, we determined the location of all gold particles contained within the reconstructed volume. From maps of their 3D-distribution, we determined that gold particles formed files that repeated at 6-7nm center-to-center apart and bent at angles measuring ~90° or ~120°. Second, we identified the tethers that linked each gold particle to the assemblies containing the chaperone. Independent of the diameter of the gold particle, tethers formed by the association of primary and secondary antibodies measured ~14nm in length. Finally, by applying the constraints represented by the repeat period, the angles and the structure of the assemblies, we identified the chaperone in unlabeled tissues as small globules spaced 6-7nm apart decorating thin filaments of the cytoskeleton. In conclusion, the high resolution in three-dimensions, the reliance on geometric constraints instead of exogenous probes and the technical simplicity are unrivaled properties of our method for studying the contribution that proteins made to normal cell homeostasis and pathological conditions.
Supported by NIH EY-04110

1083-Plat
Bind'NGO: Flexible Docking Model for Multiprotein Complexes with Intrinsically Disordered Segments
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Protein-protein interactions play an essential role in many biological processes, such asincluding DNA transcriptional regulation, signal transduction, membrane-protein trafficking and, immune response, etc. Many proteins contain flexible loops or intrinsically disordered segments, limiting the ability of atomic simulationsrigid-body docking models to sample all possible configurations and interaction modes between proteins. Here we develop a residue-level coarse-grained model for simulating multi-protein complexes with intrinsic flexibility. The intermolecular interactions are described by the transferable energy function, which was developed and applied to many weakly binding protein complexes. For the intramolecular interactions, G[[Unable to Display Character: ō]]-type potentials are derived from experimental structures. To controlinvestigate the effectdegree of flexibility of each protein, umbrella potentials along a reaction coordinate of native contacts or distance-root-mean-square deviation (dRMS) are applied with varying spring constants to control the extent of fluctuations. The model is applied to the complexes of ubiquitin and various ubiquitin binding domains. We show that the flexibility generally weakens the binding free energy, owing to the entropic penalty upon binding. However, for some cases, a weak flexible motion in the binding regions increases the binding free energies modestly. The model can be an effective tool for simulating multi-protein complexes with flexible loops or linkers, and be applied to studies of theparticularly where conformational changes upon binding are important.

1084-Plat
Adhesive Water Networks Facilitate Binding of Hydrophilic Protein Interfaces
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Saarland University, Saarbrücken, Germany.
It is well known that bulk water plays a crucial role in the biological assembly of hydrophilic surfaces. In this work, we emphasize the special molecular nature of bridging water networks in the formation of biomolecular contacts driven by electrostatic interactions. We have studied the assembly of two hydrophilic protein interfaces. Extensive atomistic molecular dynamics simulations reproducably recovered the native bound state of the Barnase:Barstar complex as seen in the crystal structure of the complex and thus give atomistic insight into the mechanism of binding. The simulations showed the structured water in the interfacial gap to play an adhesive role between the interfaces by forming a strong hydrogen bond network between the interfaces with a reduced dielectric constant compared to bulk. The role of this network is relevant already during the diffusive phase and stabilizes the early intermediate states before native contacts are formed. The convergence to the stereo-specific complex was accompanied by maximizing the interfacial water-mediation and formation of the highly hydrated stereo-specific complex. We introduce a new graph-based methodology to quantify the connectness of water networks.

1085-Plat
Crystal Structure of a Ten-Subunit Human Spliceosomal U1 snRNP at 5.5 Å Resolution
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Most eukaryotic protein-coding genes contain non-coding regions (introns) that separate those coding for protein (exons). The introns must be excised and exons spliced together from the precursor-mRNA transcript of such genes. This process (RNA splicing) is catalyzed by the spliceosome, integral to which are five RNA-protein complexes (U1, U2, U4, U5 and U6 snRNPs). A first step in RNA splicing, functioning to initiate spliceosome assembly, involves recognition of the junction between the 5′-exon and intron (5′-splice site) by U1 snRNP. Human U1 snRNP (~250 kDa) is composed of one RNA (U1 snRNA) and ten polypeptides (seven Sm proteins, U1-A, U1-70K, and U1-C). An experimental electron density map at 5.5 Å resolution enabled us to build U1 snRNA and, in conjunction with site-specific labeling of individual proteins, to place the seven Sm proteins, U1-C and U1-70K into the map. The structure reveals a hierarchical network of interactions between subunits. The seven Sm proteins interact to form a heptameric ring with a single-stranded segment of U1 snRNA leafing through its center. Sm proteins form multiple and varied interactions, with other regions of U1 snRNA as well as other protein subunits, to stabilize the structure of the particle overall. A striking feature is the amino terminus of one subunit (U1-70K), which extends over a distance of ~180 Å, wrapping around the Sm protein heptameric ring, to contact the protein U1-C. The U1-C protein is crucial for 5′-splice-site recognition. In the crystal, the zinc-finger of U1-C interacts with an RNA duplex formed between the single-stranded 5′-end of U1 snRNA and its counterpart from an adjacent complex. This unexpected interaction provides important insight into the critical role of U1-C in recognizing the precursor-mRNA transcript 5′-splice site.

1086-Plat
Nanomechanics of Clathrin Protein Shells
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The nanomechanical properties of protein shells, ranging from virus capsids to clathrin-coated vesicles, are functionally important and of notable recent interest. Using atomic force microscopy (AFM) and, more recently, quartz crystal

microbalance-dissipation (QCM-D) instrumentation, we investigate mechanical properties of intact clathrin-coated vesicles (CCVs), reassembled pure clathrin cage baskets, and single triskelia. CCVs are purified subcellular trafficking organelles of about 100 nm diameter, consisting of an outer clathrin lattice shell over a receptor- and adaptor- loaded lipid vesicle. The clathrin cages formed by self-assembly of clathrin triskelia are structurally similar to the outer shell of CCVs. From AFM and complementary QCM-D measurements, we now provide an integrative mechanical model of the clathrin protein shell and of the coupling between the clathrin coat and the membrane vesicle, in biologically relevant fluid environments. Whereas the Young's modulus of the triskelia arm is as high as a few MPa, the effective shear moduli of clathrin triskelia, clathrin baskets, and CCVs are one to two orders of magnitude smaller and vary with the buffer pH and ionic strength, as well as their proximity to a given substrate support. Such modulation of the nanomechanical properties of the assembling/disassembling protein shells may be an essential aspect of cellular function.

1087-Plat

Proline Periodicity Modulates the Self-Assembly Properties of Elastin-Like Polypeptides

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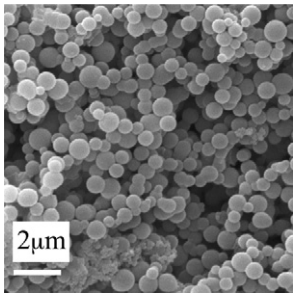
Elastin is a self-assembling protein of the extracellular matrix that provides tissues with elastic extensibility and recoil. The monomeric precursor, tropoelastin, is highly hydrophobic yet remains substantially disordered and flexible in solution, due in large part to a high combined threshold of proline and glycine residues within hydrophobic sequences. In fact, proline-poor elastin-like sequences are known to form amyloid-like fibrils, rich in β-structure, from solution. On this basis, it is clear that hydrophobic elastin sequences are in general optimized to avoid an amyloid fate. However, a small number of hydrophobic domains near the C-terminus of tropoelastin are substantially depleted of prolines. Here we investigated the specific contribution of proline number and spacing to the structure and self-assembly propensities of elastin-like polypeptides. Increasing the spacing between proline residues significantly decreased the ability of polypeptides to reversibly self-associate. Characterization of the assembly process revealed the presence of smaller colloidal droplets with enhanced propensity to cluster into dense networks, enriched in β-structure. These data strongly support a model where proline-poor regions of the elastin monomer provide a unique contribution to assembly, and suggest a role for localized β-sheet in mediating self-assembly interactions.



1088-Plat

Thrombin Flux and Wall Shear Rate Regulate Fibrin Fiber Deposition State during Polymerization Under Flow

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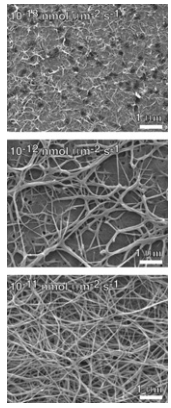
Thrombin is released as a soluble enzyme from the surface of platelets and tissue factor bearing cells to trigger fibrin polymerization during thrombosis. While isotropic fibrin polymerization under static conditions involves protofibril extension and lateral aggregation leading to a gel, factors regulating fiber growth are poorly quantified under hemodynamic flow due to the difficulty of setting thrombin fluxes. A membrane microfluidic device allowed combined control of both thrombin wall flux (10^{-13} to 10^{-11} nmol/um^2 s) and the wall shear rate (10 to 100 s^{-1}) of a flowing fibrinogen solution. At a thrombin flux of 10^{-12} nmol/um^2 s, both fibrin deposition and fiber thickness decreased as the wall shear rate increased from 10 to 100 s^{-1}. Direct measurement and transport-reaction simulations at 12 different thrombin flux-wall shear rate conditions demonstrated that two dimensionless numbers, the Peclet number (Pe) and the Damkohler number (Da), defined a state diagram to predict fibrin morphology. For Da<10, we only observed thin films at all Pe. For 10<Da<900 and Pe<100, we observed three-dimensional gels. These results indicate that increases in wall shear rate first quench lateral aggregation and then protofibril extension.



1089-Plat

Fluorescent S-Layer Fusionproteins; Reassembling Behaviour and Spectral Properties

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S-layer proteins are crystalline bacterial cell surface layer proteins. When they are isolated or genetically modified with functional moieties they are still able to reassemble spontaneously into their distinctive monomolecular lattice symmetry as originally found on the cell surface. In solution they form differently sized self-assembly products while they build crystalline nanostructured biocoatings o nsolid supports. In our case we fused the pH-dependent green fluorescent protein (EGFP) in a 1:1 stoichiometry with a truncated form of the S-layer protein SgsE from Geobacillus stearothermophilus NRS2004/3a. We investigated the formation of the 2D nanostructured monomolecular crystalline self-assembly products with the functional moiety in defined position and orientation, representing a new patterning material for biomolecular construction kits. With transmission electron microscopy (TEM) we could demonstrate that isolated SgsE-EGFP subunits build mono- and double-layered self-assembly products, forming differently sized flat sheets and cylinders with p2 lattice symmetry (a=11.9+/-0.6nm, b=14.7+/-0.6nm, g=81.2+/-1.1nm). Atomic force microscopy (AFM) was used to resolve the crystalline domain nanostructure of the fluorescent S-layer proteins on functionalised solid supports. We demonstrated the application as building blocks by coating silica particles with the fluorescent S-layer protein, fabricating in this way a pH-dependent nanostructured surface-coating. The pKa value of the pH-dependent fluorescent S-layer coating was calculated after measuring the EGFP fluorescence emission in different pH environements using flow cytometry and fluorescence microscopy. The colloidal behaviour was investigated with Zeta-potential measurements. In conclusion, these fluorescent S-layer fusion protein assemblies can be used for investigating systematically changes in pH and surface potential at the nanoscale.

AWARDS CEREMONY AND NATIONAL LECTURE

1090-Natl

Chaperonin-Mediated Protein Folding

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Chaperonins are remarkable ring assemblies that provide kinetic assistance to protein folding to the native state. They function through the action of the central cavities of their rings. The best studied such system is bacterial GroEL and its cooperating co-chaperonin GroES, which mediate productive folding of a large number of proteins in the presence of ATP. I will recount the early functional studies of chaperonins in mitochondria, carried out collaboratively with Ulrich Hartl, and describe our subsequent X-ray, EM, and biochemical studies of GroEL leading to an understanding of the structure and reaction cycle, studies carried out with Paul Sigler and Helen Saibil. The structure and mechanism studies revealed that an open ring of GroEL exposes hydrophobic surfaces that capture non-native proteins and prevent them from aggregation. In the presence of ATP, which binds cooperatively in the seven equatorial sites of an open ring, the cochaperonin ring GroES associates, producing large rigid body movements of the apical and intermediate domains of the bound, so-called *cis* ring, releasing substrate protein into a now-encapsulated central cavity that has hydrophilic walls. Folding occurs in this space in what is the longest-lived state of the reaction cycle. ATP hydrolysis gates rapid entry of ATP into the opposite ring, which both allosterically ejects the *cis* ligands and sets up a new folding active ring. I will present recent cryoEM studies of the allosteric movements driven by ATP binding, which both facilitate substrate protein binding and enable initial contact of GroES with GroEL, carried out with Helen Saibil, as well as discuss *cis* folding studies, carried out in part with Kurt Wüthrich.